PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)
	) Group Art Unit: 1633
Daniel B. Drachman	)
	) Examiner: E. Sorbello
Serial No. 09/205,096	)
	)
Filing Date: December 3, 1998	) Docket No. 01107.77737

For: **TARGETING ANTIGEN-SPECIFIC CELLS FOR SPECIFIC IMMUNOTHERAPY OF AUTOIMMUNE DISEASE**

DECLARATION UNDER RULE 132

I, Daniel B. Drachman, hereby declare:

1. I am the sole inventor of the application referenced above.

2. I have conducted experiments that demonstrate that antigen presenting cells (APCs) transduced by vaccinia virus vectors (VVV) containing genes that encode the influenza hemagglutinin (HA) antigen, Fas ligand (FasL), and truncated FADD cause effective killing of HA-specific T cells in a transgenic mouse.

3. The transgenic mouse used in the experiments has T cells that express an HA-specific T cell receptor. The HA-specific T cell receptor is expressed in approximately 50% of the T cell population of the transgenic mouse.

4. Antigen presenting cells (APCs) specific for HA-specific T cells were prepared by isolating APCs from BALB/c mice and infecting them with an attenuated vaccinia virus vector (VVV). The VVV was genetically engineered to contain three genes encoding: HA (functionally connected to mouse LAMP1), Fas ligand, and truncated FADD. Control APCs were prepared by infecting APCs with an attenuated VVV encoding two genes: Fas ligand and truncated FADD gene. The control APCs thus did not contain the gene encoding HA.

5. An HA transgenic mouse as described in paragraph 3 was injected intraperitoneally

(IP) with $5.0x10^7$ APCs specific for HA-specific T cells as described in paragraph 4. A second HA transgenic mouse was injected IP with $5.0x10^7$ of the control APCs described in paragraph 4. Two HA transgenic mice served as untreated controls and did not receive an injection of APCs.

6. HA-specific T cell killing was measured in each of the HA transgenic mice by flow cytometry. Peripheral blood lymphocytes were collected from the HA transgenic mice at two, five, and eight days after APC injections. The percentage of HA-specific $CD4^+$ T cells in the total $CD4^+$ T cell population of the mice was determined.

7. The percentage of HA-specific $CD4^+$ T cells in the mouse injected with APCs specific for HA-specific T cells was greatly reduced two days following injection compared to the mouse injected with the control APCs or untreated control mice. Specifically, the percentage of HA-specific $CD4^+$ T cells was sharply reduced to 9.4% compared to 59.2% in the mouse injected with control APCs, or 48.8% in the untreated control mice. At 5 and 8 days post APC injection the percentage of HA-specific $CD4^+$ T cells rebounded somewhat but remained reduced compared to the mouse that received the control APCs or the untreated control mice. See Table 1.

Table 1

APCs injected into mouse that express	% $CD4^+$ T cells expressing the HA receptor at 2 days	% $CD4^+$ T cells expressing the HA receptor at 5 days	% $CD4^+$ T cells expressing the HA receptor at 8 days
HA-LAMP-sig + FasL +trFADD	9.4	20.9	24.5
FasL + FADD	59.2	40.8	39.1
No APCs injected	48.8	48.8	48.8

8. Proliferation of HA-specific T cells in the transgenic mice was also assessed. The mice were euthanized twelve days after injection. Lymph node cells and splenocytes were obtained from the euthanized mice and were stimulated with HA *in vitro*. Proliferation was measured by determining the amount of radioactivity incorporated into cells one day after pulsing with ^{3}H-TdR (tritiated deoxyribothymidine).

9. We found that proliferation of HA-specific splenocytes was reduced by 3.0-3.6 fold in

the HA transgenic mouse injected with APCs specific for HA-specific T cells compared to the mouse that received the control APCs or the two untreated control mice. Similarly, proliferation of HA-specific lymph node cells was approximately 2.4-2.7 fold lower in the HA transgenic mouse injected with APCs specific for HA specific T cells compared to the transgenic mouse injected with control APCs or two untreated control HA transgenic mice. See Table 2.

Table 2

APCs that express	Counts measured in splenocytes ($x10^3$)	Counts measured in lymph node cells ($x10^3$)
HA-LAMP-sig + FasL +trFADD	34.8	48.6
FasL + FADD	106.5	129.9
No APCs injected	125.9	118.3

10. The flow cytometry and cell proliferation measurements are consistent with and support the claimed method of using APCs specific for a particular antigen-specific T cell population to activate those particular antigen-specific T cells. Co-administration of a product which is detrimental to activated T cell proliferation leads to a reduction in the antigen-specific $CD4^+$ T cell population and leads to a reduction in antigen-stimulatable proliferation of splenocytes and lymphocytes.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States; and further that false statements made willfully may jeopardize the validity of any patent issuing on an application in which the false statements were made.

1/3/02
Date

Daniel B. Drachman

Daniel B. Drachman